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14. ABSTRACT

Here we sought to understand how host biology influences the composition of skin microbes, how skin microbes influence volatiles on the skin and, in turn how skin volatiles and microbe composition influence the attractiveness of the skin to mosquitoes. Our specific aims were to sample humans and other hosts for skin microbes and volatiles. We then sequenced those samples to identify the microbes present. Our next steps are threefold. We consider how host genes influence skin microbes. We consider how microbe composition influences metabolic attributes of microbial communities (including their attractiveness to mosquitoes). Finally, we will integrate all of our types of

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Report Title

Final Report: Understanding the effects of host evolution and skin bacteria composition on disease vector choices

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Here we sought to understand how host biology influences the composition of skin microbes, how skin microbes influence volatiles on the skin and, in turn how skin volatiles and microbe composition influence the attractiveness of the skin to mosquitoes. Our specific aims were to sample humans and other hosts for skin microbes and volatiles. We then sequenced those samples to identify the microbes present. Our next steps are threefold. We consider how host genes influence skin microbes. We consider how microbe composition influences metabolic attributes of microbial communities (including their attractiveness to mosquitoes). Finally, we will integrate all of our types of data to lin host attributes to skin microbe composition, skin microbe composition to volatiles, and volatiles and skin microbe composition to attractiveness to mosquitoes. In doing so, a key focus will be on identifying the microbes and/or their volatiles that are most attractive to or repulsive to mosquitoes.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
04/14/2016	6.00	Sarah E. Council, Amy M. Savage, Julie M. Urban, Megan E. Ehlers, J. H. Pate Skene, Michael L. Platt, Robert R. Dunn, Julie E. Horvath. Diversity and evolution of the primate skin microbiome, Proceedings of the Royal Society B: Biological Sciences, (01 2016): 0. doi: 10.1098/rspb.2015.2586
04/14/2016	5.00	Julie Urban, Daniel J. Fergus, Amy M. Savage, Megan Ehlers, Holly L. Menninger, Robert R. Dunn, Julie E. Horvath. The effect of habitual and experimental antiperspirant and deodorant product use on the armpit microbiome, PeerJ, (02 2016): 0. doi: 10.7717/peerj.1605
TOTAL:	**2**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper
TOTAL:	

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

<u>Received</u>   <u>Paper</u>

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

<u>Received</u>   <u>Paper</u>

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

<u>Received</u>   <u>Paper</u>

08/31/2015 2.00 Julie M. Urban, Julie Horvath-Roth, Megan Ehlers, Daniel J. Fergus, Amy Savage, Holly Menninger, Robert R. Dunn. Hidden Life: The effect of habitual antiperspirant and deodorant product use on the armpit microbiome,
PeerJ (12 2014)

08/31/2015 4.00 Julie Horvath, Amy Savage, Julie Urban, Megan Ehlers, Pate Skene, Michael Platt, Sarah Council, Robert Dunn. Diversity and Evolution of the Primate Skin Microbiome,
Proceedings of the Royal Society B (04 2015)

TOTAL: **2**

Number of Manuscripts:

Books

Received   Book

TOTAL:

Received   Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Mary Jane Epps	0.35
DeAnna Beasley	0.35
FTE Equivalent:	**0.70**
Total Number:	**2**

Names of Faculty Supported

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics
This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in
science, mathematics, engineering, or technology fields:------ 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue
to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:------ 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):....... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for
Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work
for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive
scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Our original goals were fourfold...

1-Sample participants for skin microbes. We sampled the original number of participants proposed and then, in addition, were able to sample two additional sets of participants, one group at the NC Museum of Natural Sciences and one group associated with the Personal Genome Project. In addition, we complemented these samples with samples of non-human primates that we thought we might be able to get in the timeline of the project, but were not sure.

2-Sequence skin microbes. The first set of skin microbes was sequenced quickly (four months ago), but a second batch was delayed at the sequencing facility due to a back-up unrelated to our project. We have now sequenced both batches of human sequences, along with a third set of sequences for participants for whom we also characterized a host gene (ABC11) we thought likely to have an influence on microbial composition and ultimately attractiveness.

On the basis of the first batches of sequences we have been able to write two papers. First, we have shown that the skin microbes of the humans we have sampled are heavily influenced by the use of antiperspirant and deodorant, the use of which disfavors Corynebacterium (the slow growing bacterial taxon common in armpits and other places with apocrine glands). We are also able to show that the bacteria thought to be medically "normal," in humans are instead the Staphylococcus most favored by antiperspirant, suggesting that the commoness of these Staphylococcus is a modern (and potentially problematic) phenomenon. This paper is in revision.

Second, in comparing samples from humans to those of other primates we have shown that human skin microbes are most similar to those of other apes (which collectively differ from those of monkeys) and that the key difference between humans and other apes (other than a higher frequency of fecal microbes in the fur of apes) is the predominance of Staphylococcus on humans. Work done by other groups has shown both that Staphylococcus volatiles can attract mosquitoes and that Staphylococcus lineages are disproportionately associated with infections and other skin diseases.

Third, our original intent was to focus on metabarcoding approaches. However, we have been able to partner with colleagues who, with their own funding, are in the process of sequencing a subset of our samples using shotgun sequencing approaches. The first round of shotgun sequencing has been completed such that we can now link microbe composition to the distribution of functionally important genes and, in turn, volatiles.

3.-Consider links between skin microbes and functional assays of their attractiveness.

In doing this work, we originally intended to use traditional assays of attractiveness that have been used in the literature. However, as we considered these assays in more detail it became clear that they have a major problem. In nearly all studies of mosquito attraction to microbes to date the proportion of mosquitoes that respond is very, very small (often less than 1%) such that while a particular microbe might be more or less attractive than another, the differences are very modest and most mosquitoes in the sample are really not assayed. It took longer, but we have pioneered a new approach in which we can assay a much larger proportion of the mosquitoes in a sample. The new approach is potentially patentable and has allowed us to begin to test large numbers of samples for attractiveness. We are using two methods to do this. First, we are testing composite samples of many species of microbes from individual humans (and non-humans). Second, we have been able to culture a large number of skin microbe taxa and are using these taxa will be able to assay the attractiveness of individual taxa in isolation.

Finally, we have been able to show that in addition to the influence of host species identity (e.g., are you a chimp or a human) and host behavior (do you use antiperspirant) that a single gene has a strong influence on host microbes and microbial volatiles. We are now finishing analysis to understand which of these factors has the largest impact on host attractiveness to mosquitoes. We anticipate that these results, collectively, will result in three additional papers.

The strong influence of a single human gene on the attractiveness of hosts to microbes suggests that if we are to develop a probiotic that deters mosquitoes that its value will depend greatly on the genome of the person that uses it. Fortunately it is easy to detect which version of the key human gene a person has based on their earwax type (which is also influenced by the same key gene).

4-Our last step has been to link the attractiveness of individual hosts, their microbe composition and their individual microbe taxa to the volatiles that serve as the intermediaries of this attractiveness. In this step too we have partnered with David Karig and his collaborators so as to make this step comparable to the work on volatiles he is doing with a separate set of samples. We have now compared the relative influence of host genes, microbial composition and volatiles on attractiveness to mosquitoes. So far, the best predictor of attractiveness is the composition of microbes and host genes. However, we are considering new statistical models that might allow additional inference.

In short, we have been able to do much more ambitious work than we anticipated. The armpit ecosystem has, in some ways, proven to be more complex than anticipated. However, in other ways its influences (host genes and host behavior) have proven more simple.

Technology Transfer

There are two potential technologies associated with this project. One is a new method of assaying mosquito preference which greatly outperforms existing assays. A second would be the ability to culture bacteria that are deterrent to mosquitoes. We are moving toward the latter, but we are still in early stages.

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